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Emerging trends of long non-coding RNAs in gene activation

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The RNA world has gained increasing importance in the recent past as its role beyond coding for proteins and components of translational machinery is becoming more and more prominent. Recent studies have shown pervasive transcription throughout the genome generating a large number of non-coding RNAs (ncRNAs) but few of these RNAs have been shown to perform regulatory functions. Among the regulatory RNAs, the long non-coding RNAs (lncRNAs) form an interesting class which, with their ability to bind to a variety of targets, can play pivotal roles in cellular processes including regulation of gene expression. While lncRNAs are well known for their role in repressing gene expression, their role in gene activation is only emerging from recent studies. Here we review how the lncRNAs can mediate gene activation by a variety of mechanisms and explore their importance in biological processes.

Introduction

The central dogma of biology that information flows from DNA to RNA to protein is due for new dimensions. Recent studies have shown that most of the human genome gets transcribed although much of it does not code for proteins [1–4]. This implies that the non-coding transcripts or non-coding RNAs (ncRNAs) are the final functional forms of that part of genetic information and proteins are not the only functional molecules in the cell, as thought earlier (Table 1). All these years we have been unaware of the vast repertoire of functional RNAs produced in the cell, and this is now calling on a paradigm shift in our understanding of the central dogma (Fig. 1). This observation opens up novel evolutionary considerations about whether many of these RNAs are relics of the primitive RNA world or whether they are important parts of some yet to be discovered cellular process [5].

A large variety is seen in ncRNAs and they are found to occupy a major portion of the genomic space. Studies in the past two decades have indicated that this genome-wide transcription often makes regulatory RNAs contributing by diverse mechanisms to gene expression and regulation. These regulatory RNAs fall into two major categories on the basis of their average sizes – the small non-coding RNAs (sncRNAs) and the long non-coding RNAs (lncRNAs). The sncRNAs constitute microRNAs (miRNAs), small interfering RNAs (siRNAs), PIWI interacting RNAs (piRNAs), Alu RNAs etc. (Table 2). The sncRNAs are known mainly to repress gene

Abbreviations
ICG, interchromatin granule; lncRNA, long non-coding RNA; MEF2C, myocyte-specific enhancer factor 2C; miRNA, microRNA; MLL, mixed-lineage leukaemia; ncRNA, non-coding RNA; NEAT2, nuclear enriched abundant transcript 2; PRC2, polycomb repressive complex 2; siRNA, small interfering RNA; SRA, steroid receptor RNA activator; SRAP, steroid receptor RNA activator protein; TUG1, taurine upregulated 1.

Table 1. lncRNAs and proteins – similarities.

1. Both are final forms of genetic information: functional molecules
2. They display sequence-dependent secondary structures
3. Secondary structures of both help them in specific interactions with DNA, RNA and proteins
4. The amplitude of genetic information is dependent on rate of transcription and stability of the RNA in the case of both lncRNAs and proteins, while the latter can be further amplified during translation

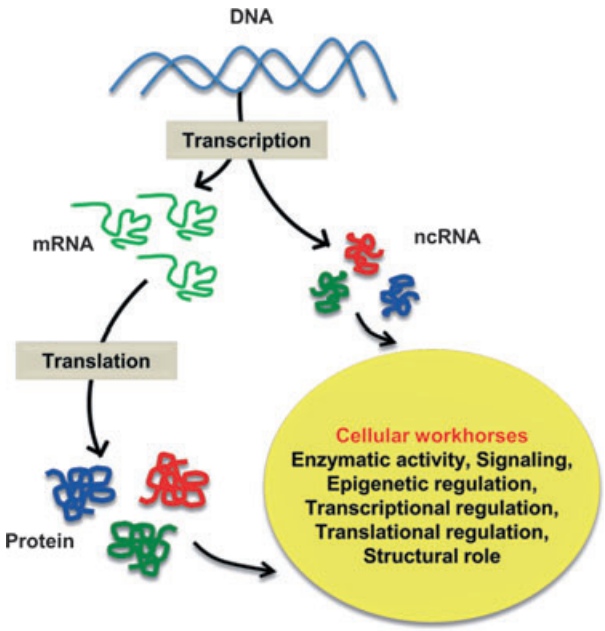

Fig. 1. ncRNA as the end product of genetic information. The new central dogma emerging from recent advances that suggest diverse functions for ncRNAs. The ncRNAs can be final functional molecules, as efficient cellular workhorses as proteins.

expression except in a few cases where dsRNA has been shown to activate genes by a process termed RNA activation whose mechanistic details still remain to be elucidated [6]. The other class of abundant ncRNAs, the lncRNAs, are > 200 nucleotides in length. Interestingly, these resemble mRNAs in many of their characteristics, e.g. transcribed by RNA polymerase II, mostly 5′ capped, spliced and polyadenylated at the 3′ end (Table 3 lists a subset of well-studied lncRNAs). Functionally, lncRNAs can be classified into three major groups: structural, repressive and activating (Fig. 2). lncRNAs can perform structural roles by providing a scaffold for the formation of paraspeckles or other structural features like the nuclear matrix [7]. Repressive lncRNAs mediate their actions in many ways such as recruiting repressive complexes at the target loci, causing transcriptional interference, allosterically modifying RNA binding proteins to subsequently inhibit transcription or preventing the formation of transcription initiation complex at the target loci. At the translational level lncRNAs can also degrade mRNAs and prevent protein synthesis [8–13]. While most regulatory RNAs discovered until now were found to be repressive in nature, recently lncRNAs have also been found to activate gene expression. In this review we discuss some of the mechanisms involved in the gene activation function of lncRNAs in vertebrates to highlight the versatility of this important component of complex genomes.

lncRNA mediates targeting of chromatin remodellers and establishment of active epigenetic marks

The transcriptional activity of a gene is dependent on whether the chromatin is accessible to the transcription machinery or not. An inactive gene has tightly packed chromatin and possesses repressive chromatin marks of H3K9me3 and H3K27me3. To activate the gene, these marks need to be replaced by marks like H3K4me3 and the accessibility of chromatin has to be increased [17]. This whole process of chromatin remodelling is carried out by chromatin modifiers in concert with chromatin remodellers. The active marks are read by the trithorax group of proteins and chromatin is made accessible for transcription [18]. One of the first signs of this process having involvement of an RNA component was in the case of dosage compensation in *Drosophila* by the RNA on X chromosome, roX, lncRNA. roX is involved in the recruitment of active histone marks on the X chromosome in male when the chromosome is hyperactivated as part of the dosage compensation mechanism (reviewed in [19]). In mammals, the first of its kind, the *HOTTIP* (HOXA transcript at the distal tip) gene, located upstream of *HOXA13* and encoding a 3.7 kb long RNA, is expressed in the distal anatomical regions of the body, as expected from its genomic position in the Hox locus [14]. RNA interference against HOTTIP resulted in the loss of active histone marks of H3K4me3 and H3K4me2 at the 5′ HOXA genes with a corresponding decrease in their expression levels. The active histone marks were found to be a result of the interaction of this RNA with WDR5 of the mixed-lineage leukaemia (MLL) complex. In order to maintain high local concentrations of this RNA, being expressed in a very low copy number, the chromosome forms a loop by intrachromosomal contacts in tissues that express HOTTIP. In this way HOTTIP recruits MLL efficiently to the 5′ Hox genes and thus maintains an active chromatin

Table 2. Non-coding RNAs.

Serial no.	Type of RNA	Size (nucleotides)	Origin	Function	Key references
1	MicroRNAs (miRNAs)	20–22	Processed by Drosha from endogenously coded pre-miRNAs	Repression by degradation of target mRNAs and translational inhibition	61–65
2	Mirtrons	~ 22	Derived from introns by a Drosha-independent pathway	Function similar to miRNAs	66, 67
3	Small interfering RNA (siRNA)	20–22	Centromeric, telomeric and other repetitive sequences	Post-transcriptional gene silencing via RNA interference mechanism	68–71
4	PIWI interacting RNA (piRNA)	26–31	Transcribed from piRNA clusters. They are lined up end-to-end and originate from the same strand	Post-transcriptional gene silencing of retrotransposons in germ line	72–74
5	Repeat associated small interfering RNA (rasiRNA)	26–31	Formed from annealed transcripts of transposable elements	Sub-class of piRNAs, involved in maintenance of heterochromatin, regulation of transcription from repetitive sequences	59
6	tRNA derived RNA fragments (tRFs)	15–25	Derived from processing of tRNAs	Translational repression; control of silencing activities of miRNA and siRNA	75, 76
7	SnoRNA-derived RNA (sdRNA)	17–30	Small nucleolar RNAs are processed to give sdRNAs	Putative role in gene silencing	77
8	Centromere associated RNA (crasiRNA)	~ 40	Transcribed from satellite repeatsin centromere	Maintenance of centromere integrity and repressive histone marks in that region	78
9	AluRNA	~ 300	Transcribed from Alu retroposons	Cause repression by forming transcriptionally inert complexes with RNA pol II	79, 80
10	Transcription initiation RNAs (TiRNA)	17–18	Transcribed from sequences immediately downstream to transcription start sites of many actively transcribed genes	Hypothesized to position nucleosomes and cause gene activation	81
11	Small activating RNA (saRNA)	~ 19	Synthetic small dsRNA exogenously injected into cells. Endogenous sources yet unknown	Activation by mediating loss of lysine-9 methylation on histone 3 at the target sites	6, 81
12	Promoter-associated short RNAs (PASRs)	< 200	Transcribed from 5′ UTRs of genes	Proposed to mediate both activation and repression	82
13	AAGAG NuMat RNA	~ 3 kb	AAGAG satellite repeats	Structural maintenance of nuclear matrix	27
14	Long non-coding RNAs (lncRNAs)	> 200	Transcribed from their respective genes	Act by diverse mechanisms to both repress and activate target gene expression	See Table 3

state [14] (Fig. 3A). Subsequent studies involving Mistral (Mira). an RNA from the spacer region between *Hoxa6* and *a7,* showed how lncRNA can mediate recruitment of MLL complex by formation of an RNA–DNA hybrid resembling a transcription bubble at its own locus [20]. The 3′ region of the RNA which forms a stem-loop structure, called the activator binding domain, is responsible for binding to SET domain of MLL1 which in turn has the capability to bind to ssDNA. This creates a high local concentration of the activating MLL complex and enables specific *cis*-targeting of the MLL complex to the *Hoxa6* and *a7*. This also leads to the establishment of active H3K4me3 marks on the locus. These findings highlight the importance of secondary structures in lncRNA function. The stem loop of Mira RNA bound to the regulatory protein MLL1 is the key feature that brings specificity in its function (Fig. 3B). A unique aspect of

Table 3. Some well-studied long non-coding RNAs.

Serial no.	Name of lncRNA	Length	Function	Key references
1	Xist	17 kb (mouse), 19 kb (human)	Mediates X chromosome inactivation	83
2	Air	108 kb	Silences Slc22a3, Slc22a2 and Igf2r genes by recruiting the G9a histone methyltransferase	9
3	HOTAIR	2.2 kb	Represses *HOXD* expression by recruiting PRC2	8
4	Kcnq1ot1	90.5 kb	Silences the Kcnq1 domain by recruiting G9a methyltransferase and PRC2	84
5	TUG1	6.7 kb	Targets the E2F1 controlled genes to repressive polycomb bodies	24
6	NRON	0.8–3.7 kb	Represses NFAT activity by regulating its nuclear trafficking	85
7	SCA8	< 32 kb	Triplet expansion in this RNA is associated with spinocerebellar ataxia (SCA)	86
8	MENε/NEAT1	4 kb	Maintains structure and integrityof nuclear paraspeckles	87
9	MENβ	23 kb	Maintains structure and integrity of nuclear paraspeckles	7
10	HOTTIP	3.7 kb	Maintains active chromatin at the 5′ *HOXA* genes	14
11	Mira	798 nt	Activates *Hoxa6* and *Hoxa7* by recruiting MLL complex	20
12	NEAT2	8.7 kb	Targets genes to active functional compartment of the nucleus – the interchromatin granules	23, 24
13	Tsix	40 kb	Antagonizes Xist RNA to maintain one active X chromosome	88
14	XACT	251.8 kb	Coats one of the X chromosomes to keep it active	21
15	Bvht	590 nt	Hypothesized to activate genes by sequestering away repressive protein SuZ12	30
16	lincMD1	Not known	Decoy to sequester miRNAs to derepress miRNA targets	16
17	ncRNA-a7	600 nt	Interacts with the mediator complexof the transcription complex to enhancegene expression	36
18	Evf-2	3.8 kb	With the help of Dlx2 and MECP2, activates *Dlx5* and *Dlx6*	15, 33
19	SRA	0.7–1.5 kb	Coactivator for steroid receptor complex, VitD and MyoD receptors	44
20	Meg3	1.6 kb	Tumour suppressor, enhances p53 expression	89

RNA being the functional product of a gene is its high local concentration. This makes such RNAs highly specific for their action in *cis*. Another example of activation by *cis*-acting RNAs comes from a recent report of an RNA, X active coating transcript (XACT), coating in *cis* the active X chromosome of mammals [21]. As the role of this RNA is to keep the X chromosome active, it is likely that it also mediates recruitment of activating proteins like Mira and HOTTIP. These observations provide a novel mechanism of how lncRNA can activate genes efficiently and specifically by its ability to bind epigenetic regulators of the genome and its proximity to target loci. They also bring out the advantage of RNA as regulator and how it can exert its effect on specific genes without the need to get translated, thereby being fast and economical for the cell (Table 4).

lncRNA mediated targeting of genes to active nuclear compartments

The eukaryotic nucleus contains sub-nuclear compartments like the nucleolus, interchromatin granules (ICGs), PcG bodies, cajal bodies, perinucleolar compartment and PML bodies that spatially define the functional state of the loci associated with them (reviewed in [22]). Little is known, however, about how genes translocate from one domain to another within the nucleus when their functional status alters. A search for polyadenylated transcripts enriched in the nuclear compartment revealed nuclear enriched abundant transcript 2 (NEAT2) that was found to have a close association with the SC35 splicing compartment of the nucleus [23]. A recent report shows that NEAT2 lncRNA is involved in the relocation of genes from the inactive polycomb bodies to the active ICG cluster [24,25]. The execution for this translocation depends on the status of lysine-191 methylation of Pc2. While the methylated Pc2 interacts with taurine upregulated 1 (TUG1) lncRNA, the unmethylated form binds to the NEAT2 lncRNA. TUG1 lncRNA associates with polycomb repressive complex 2 (PCR2) [26], and therefore when bound to methylated Pc2 relocates growth-control genes to repressive compartments. On the other hand, NEAT2 is an activating lncRNA that

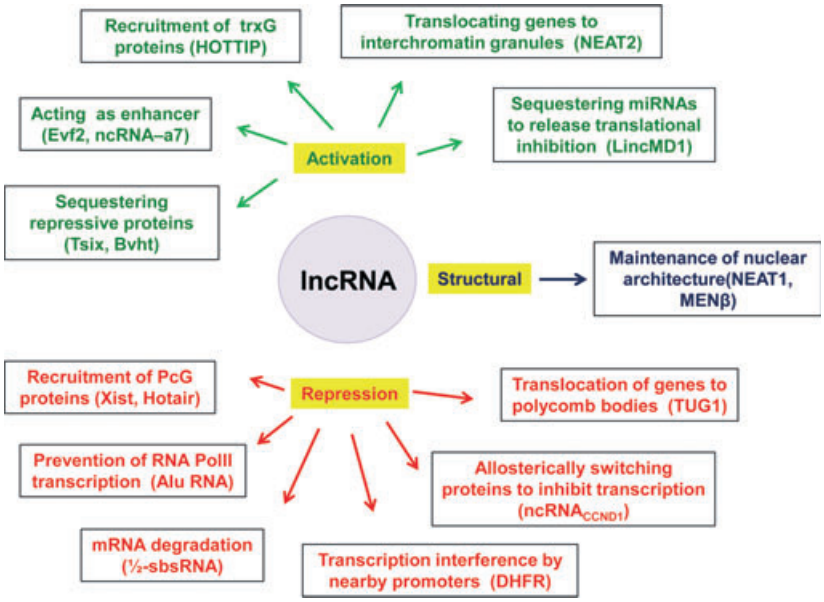

Fig. 2. Types of lncRNA. lncRNAs fall into three functional categories: activating, repressing and structural. Each arrow indicates a variety of mechanisms along with an example in each category.

associates with proteins like MLL and thereby leads target genes to the ICGs (Fig. 3C). That this relocation is dependent on the lncRNAs is confirmed by the observation that loss of these RNAs causes a failure in the corresponding sub-nuclear localization of the target genes and thus disrupts the gene regulation system. This report shows an example of how lncRNA can have an influence on the sub-nuclear dynamics and thus upon coordinated gene regulation. The study also opens up a possibility of lncRNAs having a role in nuclear architecture and its functional dynamics. We have recently shown transcripts from satellite repeats to have an essential role in the integrity of nuclear architecture [27]. Functional analysis of lncRNAs identified from high throughput studies are needed to better understand this new function of the ncRNAs.

lncRNA as a decoy to counter repression

The eukaryotic genome, by default, is kept in a repressive state. In order to activate gene expression, silencing components need to be taken away and activating components need to be brought in at the target site. Owing to their capability of efficiently and specifically binding to proteins and other RNAs, lncRNAs can act as decoys to sequester away repressive components to allow activation to take place. One of the examples for this comes from the study of dosage compensation in mammals where one of the X chromosomes randomly undergoes inactivation in females. How a cell ensures only one (in)active X chromosome depends on the Tsix lncRNA which is transcribed anti-sense to Xist and RepA that are themselves lncRNAs transcribed from the inactive X [28]. Tsix, owing to its higher copy number, its ability to bind to the repressive PRC2 complex and its sequence being anti-sense to RepA, sequesters away, like a decoy, the repressive RepARNA–PRC2 complex, thus deciding the *cis* chromosome to be the active one [29].

More recently, braveheart (*Bvht*) lncRNA was found to interact with SUZ12, a member of PRC2. Bvht is demonstrated to activate gene expression. It is hypothesized that this interaction prevents PRC2 from acting on the target loci [30]. It has been reported earlier that many lncRNAs interact with chromatin modifying complexes but recent results indicate that these RNAs may have a role in addition to the repressive one [26]. It is also possible that lncRNAs trap and maintain a pool of repressive proteins and release them upon getting signals to repress genes.

lncRNAs have also been shown to act as decoys for miRNAs and prevent them from causing translational inhibition of their targets. The linc-MD1 lncRNA has been shown to act as a competing endogenous RNA for miR-135 and miR-133 which are negative regulators of genes involved in myogenesis, myocyte-specific enhancer factor 2C (MEF2C) and mastermind-like-1 (MAML1), respectively [16] (Fig. 3D). Similar sequestering is done by RNAs from pseudogenes that sequester away miRNAs from binding to their functional counterpart and thus can be regarded as activating

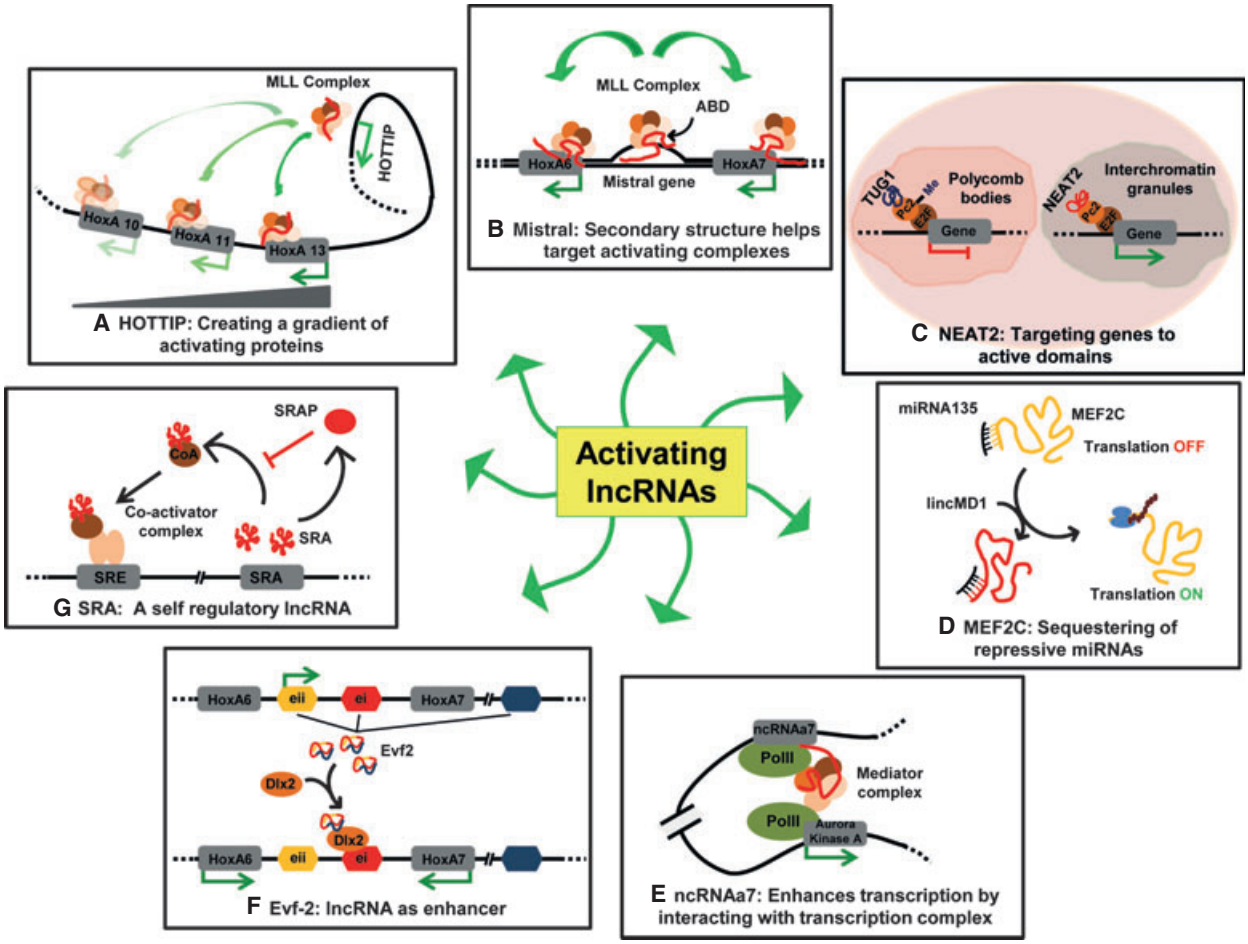

Fig. 3. Diverse mechanisms of gene activation by lncRNAs. (A) The HOTTIP RNA in its domain of expression activates Hox genes with the most proximal hox gene getting activated to the highest levels followed by diminishing levels in distal hox genes. This phenomenon is aided by the looping of chromatin in that region. (B) The activator binding domain (ABD) of the Mistral RNA, with its ability to interact to the MLL1 protein of the MLL complex, recruits the activator complex to the adjacent hox genes HOXA6 and HOXA7. (C) TUG1 and NEAT2 lncRNAs bind to Pc2 protein. While TUG1 binds the methylated form and directs the target genes to repressive polycomb bodies, NEAT2 binds to the unmethylated form and takes the genes to the active compartment of the nucleus, the interchromatin granule. (D) miRNA135 targets MEF2C mRNA for degradation and thus prevents MEF2C translation. The lincMD1 sequesters away these miRNAs thus allowing the translation of MEF2C protein. (E) The *ei* enhancer gets transcribed to give the *Evf-2* RNA, which binds to Dlx2 to recruit it to the *Dlx5/6* locus leading to their activation. (F) The ncRNA-a7 interacts with the mediator proteins of the transcription machinery and enhances the transcription of target locus aurora kinase A. (G) The steroid receptor activator RNA (SRA), a part of the steroid receptor coactivator complex (CoA), is regulated by self-coded peptides called steroid receptor activator proteins (SRAPs).

lncRNAs. The PTEN mRNA is prevented from repression by PTEN1 RNA from its pseudogene [31]. All pseudogenes may not therefore be evolutionary dead ends but instead may be products of positive selection for gene regulation. It will be interesting to look for lncRNA pseudogene transcripts in the genome acting as competing endogenous RNAs by looking for miRNA target sites in them.

In a recent report, lncRNAs have been shown to maintain heterochromatin–euchromatin barriers, presumably by evicting or sequestering away repressive proteins like Swi6 in fission yeast [32]. Surprisingly, transcription activity seems to be the sole requirement for this barrier maintenance without any specific sequence requirement. It is worthwhile probing into the barrier regions in different model systems to look for the role of lncRNA in restricting the spread of heterochromatin and thereby keeping nearby genes active.

lncRNAs as enhancers

Between the homeodomain protein family members *Dlx5* and *Dlx6* falls the ultraconserved region that consists of enhancers *ei* and *eii* of which *ei* gets tran-

Table 4. Regulatory RNAs – an advantage over proteins

1. Specificity can be of ultimate degree in the case of lncRNAs due to availability of base pairing
2. RNA as a functional molecule is a faster and a cheaper option for cells as translational time and cost are avoided
3. Lack of coding restraint gives more space for sequence diversity
4. Not essential for survival and thus serve as a sink for many mutations, thereby fine-tuning gene regulation and accelerating evolution

scribed as Evf2 RNA that can transactivate *Dlx5* and *6* in cooperation with Dlx2 protein [15] (Fig. 3E). However, upon knockout of the Evf2 transcript, an increase in the levels of Dlx5 and 6 was seen [33]. Upon ectopically providing the Evf2 RNA at lower concentrations, *Dlx5* expression decreased while *Dlx6* level did not change indicating a repressing function of Evf-2 on *Dlx5* in *trans*. However, at a higher Evf2 concentration, both Dlx5 and Dlx6 increased. The inability of ectopic Evf2 transcripts to decrease Dlx6 in *Evf2* mutants suggests that Evf2 reduced Dlx6 expression through anti-sense competition in *cis*. Further studies showed the interaction of two proteins with opposite functions – Dlx2 and MECP2 interact with the enhancer region in the presence of Evf2 RNA. Detailed analysis of these interactions led to the hypothesis that the Evf2 RNA may have the capability to recruit both repressive and activating proteins to the enhancer region and thus bring about corresponding changes in gene expression [33]. Although it does not bring clarity to the mechanism, *Evf2* still remains unique as it is the first among its kind to function at both DNA and RNA levels as an enhancer and/or repressor.

Interestingly, many more studies are being published that report transcribing enhancers. In one of them several neuronal enhancers have been shown to produce lncRNAs < 2 kb in length [34]. Similarly, a genome-wide search for transcripts using the GENCODE annotation showed the presence of ~ 3000 putative ncRNAs of which many were shown to function as positive regulators as knockdown of some of these lncRNAs, namely ncRNA-a1 and a2, resulted in decreased expression of adjacent genes [35,36]. Also, depletion of ncRNA-a7 resulted in a decrease in the gene expression of aurora kinase A, a gene that is located 6 Mb from the ncRNA locus. These findings highlight the potential of lncRNAs to act as gene-specific enhancers mediating their effects close by in *cis* or at a distance. Furthermore, the ncRNA-a7 was shown to interact with mediator proteins MED1 and MED12 of the transcriptional machinery [37]. The lncRNA locus loops to interact with its target locus to mediate transcription activation. It remains to be seen how specificity is achieved in this process. It is likely, though, that secondary structure formation and thereby stabilization of the transcriptional machinery or increasing local concentrations of the PolII play a role in this process (Fig. 3F). Recently, it has been shown that p53 which is a transcription factor can also enhance transcription by binding to enhancer regions which are transcribed to mediate long-distance p53-dependent gene regulation [38]. Finally, ligand induced transcription of enhancers has also now been reported [39]. Although these studies improve our understanding of how enhancers may be functioning, the exact mechanism remains to be established.

Tissue specificity is the key characteristic of enhancers. This is in some cases mediated by tissue-specific transcription factors [40]. Another likely means to bring in this specificity may be tissue-specific transcription of these enhancers. The enhancer RNAs, then, interact with activating proteins and/or PolII machinery to increase the proximity of these proteins to the target loci by a looping mechanism. It is possible that, when one enhancer targets many genes, they may all be coming together physically to enable efficient loading of polymerase. It has also been seen that in some cases the transcript *per se* may not be playing a major role in activation and the act of transcription (e-transcription) alone may be more important [32,41]. It is important to investigate the potentially distinct contribution of enhancer RNA and e-transcription at different loci and to see if this mechanistic difference associates with specific features linked to the two classes of loci.

A self-regulating lncRNA acts as a coactivator

Activator proteins, in general, function in complexes which also include coactivator complexes. Apart from proteins, one of the coactivator complexes, the steroid receptor coactivator complex, showed the presence of an RNA component, steroid receptor RNA activator (SRA) [42,43]. SRA also coactivates other transcription factors such as MyoD and Vit-D-R [44]. It has been found to have 11 stem-loop structures and mutations that altered the secondary structures and severely diminished its coactivator function [45]. This supports the emerging view that lncRNAs mediate their activity not only in a sequence-dependent manner but also in a structure-dependent manner. The uniqueness of SRA lncRNA is the fact that it also gets translated. Recently many isoforms of SRA have been reported that originate by alternative splicing or alternative

promoters that have been known to code for short peptides termed steroid receptor RNA activator proteins (SRAPs) that bind to one of the sub-structures of SRA and prevent it from binding to the coactivator complex [46–48]. These studies point out a negative regulation of the lncRNA function in a feedback-loop-like mechanism. The complexity of the function of SRA, however, emerges from the finding that it helps CTCF to function as an insulator protein and repress gene expression, indicating a negative role for this lncRNA [49]. The SRAPs have also been shown to enhance androgen receptor activity upon their ectopic expression [50]. These observations suggest that the RNA and protein may be having varying functions depending on cell/tissue type and context. Despite this complexity, SRA is the first reported lncRNA to function at both RNA and protein level and to regulate itself by translating into self-binding peptides. The studies also point out a clear role of secondary structure in SRA function. A detailed secondary structure determination for lncRNAs may identify diverse lncRNA–protein, lncRNA–RNA interactions and help us understand the mechanisms of lncRNA function.

lncRNAs abound in the human genome

Much of the human genome codes for ncRNAs while a tiny fraction codes for proteins [50a,50b]. As a huge chunk of the genome is transcribed, with a lot of transcripts with unknown function, it was attractive to take up transcriptome analysis with novel approaches to find lncRNAs across the genome. Two such approaches identified over 4000 novel lncRNAs. One such approach was by looking into specific chromatin signatures that mark actively transcribed genes outside the protein coding regions of the genome [51]. The other search was based on the GENCODE annotation with the modification that transcripts overlapping protein-coding genes were excluded from the list [36]. With the discovery of these lncRNAs, it is time for probing into their functional details and mechanism of action. It has been observed that many of these lncRNAs are colocalized with protein-coding genes in the genome. Many such loci have been identified in the mouse brain [53]. Some loci that escape X chromosome inactivation have also been found to have such pairs of coding and non-coding genes. Interestingly, these non-coding transcripts localize at their own genomic loci, hinting at their possible role in *cis* by helping the regions escape X-inactivation [52]. Further, a number of lncRNAs that have their genomic loci conserved with respect to the adjacent coding gene(s) are enriched in phastCons (phylogenetic analysis with space/time models) sequences – elements that are evolutionarily conserved (Cons) across species – found by a multiple sequence alignment approach [54,55]. The genomic pairing of coding and non-coding genes signifies coevolution and in many cases co-regulation. These phastCons sequences have also been shown to be enriched in predicted RNA secondary structures many of which have been found in functional lncRNAs [56], together signifying their selective evolution.

lncRNAs – the road ahead

The field of lncRNAs is at a very interesting juncture where we know many lncRNAs across the genome and it is time to look ahead. Many of the recently discovered lncRNAs function in *trans* indicating the involvement of functional protein partners [36]. The RNA Binding Protein DataBase provides a list of many interesting RNA binding proteins. The list should be expanded to find, on a global scale, proteins assisting lncRNAs and thereby reveal the cellular networks in which these RNAs are involved [57,58]. Further, lncRNAs that form characteristic secondary structures can be exploited to decipher their interacting partners and thereby their cellular functions. The existing studies on lncRNAs also bring up new questions. A major part of the human genome is composed of repeat sequences and many reports suggest transcription of these repeat regions. It will be interesting to look into repeat RNAs too as most studies until now have ignored this aspect. The report on heterochromatin–euchromatin transition regions getting transcribed hints that not all transcription from the repeats may be for heterochromatin formation, as is the case with repeat associated siRNAs [32,59]. Further, as it is now established that lncRNAs are essential for gene regulation in the nucleus, this calls for a thorough search for RNAs outside the nucleus in other cellular processes like signalling, cell membrane dynamics etc. Also, as these RNAs have been seen to be important in nuclear architecture, there opens up a possibility of their involvement even in the maintenance of cytoskeleton and extracellular matrix too [27]. Cell division is a complex process involving nuclear envelope breakdown and a shutdown of all transcription and translation. A question that has always been intriguing is how the parental identity is restored in daughter cells. How does the cell reactivate the same set of genes as in the parent? We have seen the ability of lncRNAs to activate gene expression. So do these activating lncRNAs remain associated with the mitotic chromosomes so that gene activation takes place as soon as

the cell enters interphase? It will be exciting to look into the RNA repertoire during cell division.

A decade ago we knew ncRNAs as siRNAs and miRNAs which are tools for gene repression in the cell. The definition of ncRNA has now changed with the discovery of lncRNAs that function as structural, repressive and, as highlighted in this review, activating components. lncRNAs are comparable to proteins in many of their features which include the ability to have secondary structures and interaction with DNA, RNA and protein molecules. In fact, their ability to base-pair with nucleic acids gives ultimate specificity for gene regulation. Further, these RNAs in concert with proteins can bring about functional and regulatory diversity in the cell thus making lncRNAs a versatile regulatory molecule of the cell. The novelty of lncRNAs lies in their ability to activate gene expression, a property not common in regulatory RNAs. It is therefore likely to be exciting to search for a role of lncRNAs during early developments after fertilization activation of a number of genes takes place in a regulated manner. Activating lncRNAs hold potential in therapeutics too. Cancers, for example, show downregulation of tumour suppressor genes. An initiative should be taken to explore the possibility of targeting lncRNAs for restoring the expression of those genes to normal levels. There are also diseases that are caused by haplo-insufficiency of genes [60]. It may be relevant to upregulate the endogenous gene to help alleviate disease/disease symptoms. Although precise targeting and delivery still remains an issue as with other gene therapy approaches, specificity of these RNAs and their low dosage requirements may give lncRNAs an edge over other methods.

The global picture of gene regulation has changed since the emergence of RNA as a regulatory molecule. lncRNAs constitute a major proportion of this RNA world. Thus, with lncRNAs as an example we suggest that RNA, as a versatile regulatory molecule, is as robust and diverse as proteins. This therefore adds a new angle to the central dogma of biology where RNA was just a passive messenger of the genetic information from the DNA to be coded into proteins. Now it belongs to a class of molecules that has independent and widespread functions in the cell (Fig. 1). From the pace at which the RNA world is expanding, ncRNAs including lncRNAs are emerging as major regulatory and structural components of cellular processes. Major expansion of lncRNAs as activators of gene regulation by a variety of mechanisms is already changing our picture of the nuclear process and this trend is set to continue.

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